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CITATION:

Yamazaki, Yuji ...[et al]. Histological Comparisons of Intestines in Parasitic and Nonparasitic Lampreys, with Reference to the Speciation Hypothesis. *Zoological Science* 2001, 18(8): 1129-1135

ISSUE DATE:

2001-11

URL:

<http://hdl.handle.net/2433/108605>

RIGHT:

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# Histological Comparisons of Intestines in Parasitic and Nonparasitic Lampreys, with Reference to the Speciation Hypothesis

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**ABSTRACT**—Histological comparisons of intestinal internal structures were made for the monophyletic lamprey group comprising parasitic *Lethenteron japonicum*, and nonparasitic *L. kessleri* and the northern form of *L. reissneri*, in order to verify the speciation hypothesis that the nonparasitic species have been derived from a congeneric parasitic species. In the larval stage of each species, the mucosal epithelial cells were regularly arranged around an inner layer of intestine, including the typhlosole. At the metamorphosed stage, *L. japonicum* possessed functional mucosal folds, reflecting an adaptive change for parasitic feeding after metamorphosis. The two nonparasitic species, in which feedings are absent after metamorphosis, also exhibited mucosal folds albeit in a degenerative condition, indicating the likely presence of functional or at least rudimentary mucosal folds in an ancestral parasitic species. This finding supports a previously advocated direction of speciation in lamprey satellite species, namely nonparasitic *L. kessleri* and the northern form of *L. reissneri* speciated from ancestral stocks of parasitic *L. japonicum*.

**Key words:** life-history evolution, metamorphosis, maturation, degeneration, rudimentary, character

## INTRODUCTION

Since elucidation of the speciation process is one of the foremost interests in biology, many authors have attempted to establish systematics, phylogenies and phenotypic character evolution, using various approaches including genetic, morphological, ecological, physiological and biogeographical data (Futuyma, 1998; Howard and Berlocher, 1998). Because lampreys, usually designated as agnatha, have been considered as a representative of primitive vertebrates, as well as hagfishes, they have received special attention in speciation studies and other diverse fields (Hardisty and Potter, 1971b; Hardisty, 1986; Yamazaki and Goto, 2000).

Many lamprey genera include several species, variously characterized by parasitic-diadromous or nonparasitic-fluvial life-cycles (Hubbs and Potter, 1971; Potter, 1980). Such species' assemblages are generally called satellite species

(Vladykov and Kott, 1979), the nonparasitic-fluvial forms being thought to have been most likely derived from congeneric parasitic-diadromous species (Hubbs, 1925; Zanandrea, 1959; Hardisty and Potter, 1971b; Vladykov and Kott, 1979; Potter, 1980; Beamish, 1985). Evidence for the direction of speciation has been based on differences between the two forms in some phenotypic character states, for example, the internal structure of the intestine (Battle and Hayashida, 1965; Hardisty *et al.*, 1970; Youson, 1981; Hilliard *et al.*, 1983) and buccal gland (Baxter, 1956; Hardisty and Potter, 1971a) between the two forms. Almost all studies for this subject have paid attention to the satellite species comprising *Lampetra fluviatilis* and *L. planeri*, except for some instances for the satellite species comprising *L. ayresii* and *L. richardsoni* (Hardisty and Potter, 1971b; Beamish, 1985, 1987; Hardisty, 1986; Docker *et al.*, 1999).

Although only three Far East *Lethenteron* species, parasitic-anadromous *L. japonicum* and nonparasitic-fluvial *L. kessleri* and *L. reissneri*, have been formally described (Iwata *et al.*, 1985; Renaud, 1997), recent allozyme analyses have disclosed two genetically divergent groups (northern and

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southern forms) within *L. reissneri* distributed in Japan and Korea (Yamazaki and Goto, 1996, 1998; Yamazaki *et al.*, 1999). These four *Lethenteron* forms should be regarded as discrete species, owing to the existence of reproductive isolation between all possible pairs of the species at sympatric sites, confirmed by allele displacement between the pairs (Yamazaki and Goto, 1998). Based on a phylogenetic analysis, *L. japonicum* appears to be closely related to *L. kessleri* and the northern form of *L. reissneri*, whereas the southern form of *L. reissneri* is greatly divergent from the other three, suggesting that the former three species are monophyletic and therefore "true" satellite species (Yamazaki and Goto, 1998).

Recently, some authors advocate that the reconstruction of character states, enabling the development of a phylogenetic tree, aids the determination of speciation direction (e.g. Felsenstein, 1985; Wells and Henry, 1998; Omland, 1999). However, because an informative outgroup had not been established in the above-stated allozyme analysis, the direction of speciation of the satellite species was not able to be determined based on that method. In the present study, giving attention to phenotypic character states as mentioned in the previous lamprey speciation studies, the histological characteristics of the intestine of *L. japonicum*, *L. kessleri* and the northern form of *L. reissneri* were compared, in the hope of verifying the direction of speciation.

## MATERIALS AND METHODS

Lamprey samples, collected from Japanese waters, include each life-history stage, i. e., larval, completely metamorphosed (corresponding stage after 7, *sensu* Potter *et al.*, 1981) and mature adult, for *Lethenteron japonicum*, *L. kessleri* and the northern form of *L. reissneri*, and both feeding and upstream migration adult stages for *L. japonicum* (see Appendix). All identifications were based on morphological features as reported by Iwata *et al.* (1985), and supported by diagnostic allozymic alleles on 11 loci (*AAT-1\**, *G6PDH\**, *GPI-2\**, *IDHP-1\**, *2\**, *3\**, *4\**, *MDH-3\**, *4\**, *MEP-1\** and *PGM\**), according to Yamazaki and Goto (1996, 1998), owing to the difficulty in distinguishing between the sympatric northern and southern forms of *L. reissneri* (Yamazaki and Goto, 1997).

Metamorphosed individuals of *L. japonicum* (as feeding adults) were collected from the Saru River, Hokkaido, Japan. Under experimental conditions, these were cultured for about six months in freshwater, followed by about four months in seawater (water temperature 14°C), during the latter time being parasitic on *Tribolodon ezoe* (Cyprinidae). Subsequently, these adults were fixed and deposited in the Laboratory of Marine Zoology, Faculty of Fisheries, Hokkaido University, Hakodate (HUMZ).

All of the lamprey samples were fixed in 10% phosphate buffered formalin. From each specimen, a 10 mm length of posterior intestine, located under the anterior edge of the first dorsal fin, was removed and preserved in 80% ethyl alcohol. Intestinal nomenclature follows Youson (1981). For histological observations, intestine samples were fixed with Bouin's fixative for 3 hr, dehydrated in a butyl alcohol series and embedded in paraffin. Serial sections, cut at 8 µm thickness, and stained with Delafield's hematoxylin and eosin, were mounted on glass slides with 0.005% poly-L-lysine (Wako Co. Ltd.).

For intestine samples at metamorphosed stage, measurements were carried out as follows: the height of the mucosal fold, the verti-

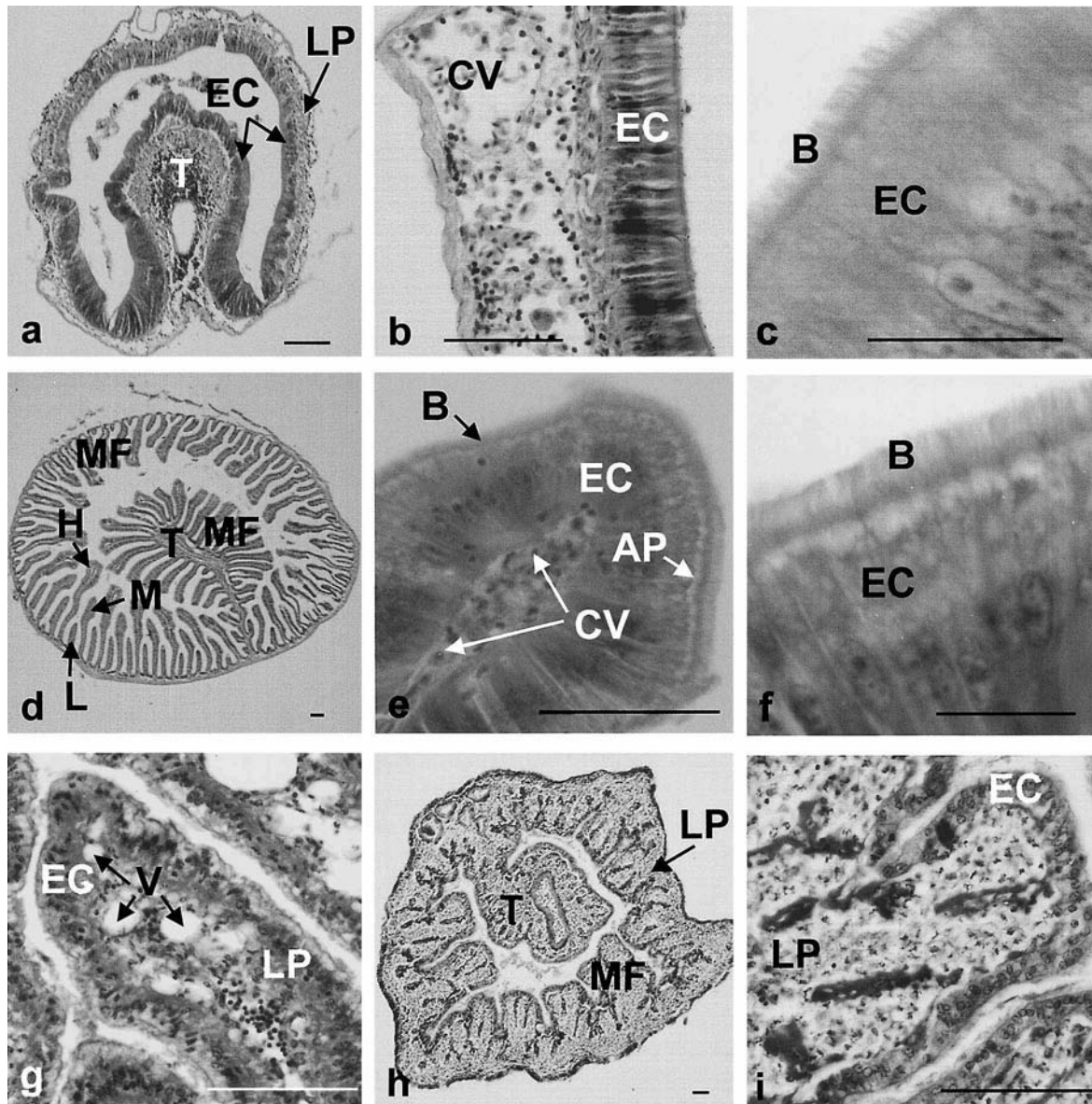
cal height from the apex of the mucosal epithelial cells of the fold to the external margin of the intestine, and that of the base, the vertical height from the apex of the mucosal epithelial cell at the middle position between the former fold and the right one to external margin of the intestine. Measurements were made for 3–15 folds in every intestine sample under a dissecting microscope to the nearest 0.01 mm using a micrometer. All measurements of folds are shown as proportions of the base height.

## RESULTS

In the larval stage of each species, the intestinal mucosal epithelial cells, being columnar with brush borders on their apices, were regularly arranged around the inner layer of the intestine, including the typhlosole (Figs. 1a–c, 2a–c, 3a–c). Well-developed networks of capillary vessels were found in the intestinal lamina propria mucosae in larvae of each species.

In the metamorphosed and feeding adult stages of *L. japonicum*, three types in height of mucosal folds were observed (Fig. 1d): higher folds revealed 6.10–12.00 (n=5, average 8.76) times in male and 4.00–12.43 (n=5, average 7.03) times in female as high as the base, middle folds revealed 4.10–11.33 (n=5, average 6.43) times in male and 3.29–11.00 (n=5, average 5.27) times in female as high as the base, and lower fold revealed 2.00–4.25 (n=5, average 2.69) times in male and 1.25–6.43 (n=5, average 2.64) times in female as high as the base. These three types of folds were mainly arranged in the order, high, low, middle, low, around the internal cavity of intestine. Well-developed capillary networks of capillary vessels were found in the internal lamina propria mucosae, including mucosal folds (Fig. 1e). The intestinal mucosal epithelial cells of all mucosal folds observed have brush borders on their apices, and were regularly arranged around the inner layer of the intestine (Fig. 1e–f). On the mucosal epithelial layer, apical pits were observed at the just inner of brush borders in the feeding adult stage of *L. japonicum* (Fig. 1e). Compared with above, specimens at the upstream migration stage had a slightly reduced height of mucosal folds as a whole, although it was difficult to measure the folds because of their curved and complicated shapes. The lamina propria mucosae, in which many vacuoles existed, were somewhat disordered, and included some collapsed capillary networks (Fig. 1g).

At the metamorphosis stage in both of *L. kessleri* and the northern form of *L. reissneri*, only one type in height of mucosal folds was observed (Figs. 2d–e, 3d–e): these revealed 1.25–2.31 (n=3, average 1.46) times in male and 1.25–2.90 (n=4, average 1.89) times in female as high as the base in *L. kessleri*, and 1.33–4.44 (n=7, average 2.40) times in male and 1.54–3.40 (n=5, average 2.42) times in female as high as the base in the northern form of *L. reissneri*. These folds, in which some vacuoles existed, were arranged around the internal cavity of the intestines, excluding the typhlosole. Compared with their respective larval stages, these specimens had mucosal epithelial cells of disordered arrangement, a few brush borders, and collapsed capillary networks in a disor-



**Fig. 1.** Transverse sections of the posterior intestine of parasitic-anadromous species, *Lethenteron japonicum*. a; Larval stage. b; Enlargement of "a" showing mucosal epithelial cells (EC) and lamina propria mucosae (LP). c; Enlargement of b showing mucosal epithelial cells. d; Metamorphosed stage. e; Enlargement of mucosal fold (MF) at feeding adult stage posterior intestine. f; Enlargement of e showing mucosal epithelial cells. g; Enlargement of mucosal fold at upstream migration stage. h; Maturation stage. i; Enlargement of h showing mucosal fold. Scale bars = 50  $\mu$ m, except c and f indicating 10  $\mu$ m. AP, apical pit; B, brush border; CV, capillary vessel; H, high mucosal fold; L, low mucosal fold; M, middle mucosal fold; T, typhlosole; V, vacuole.

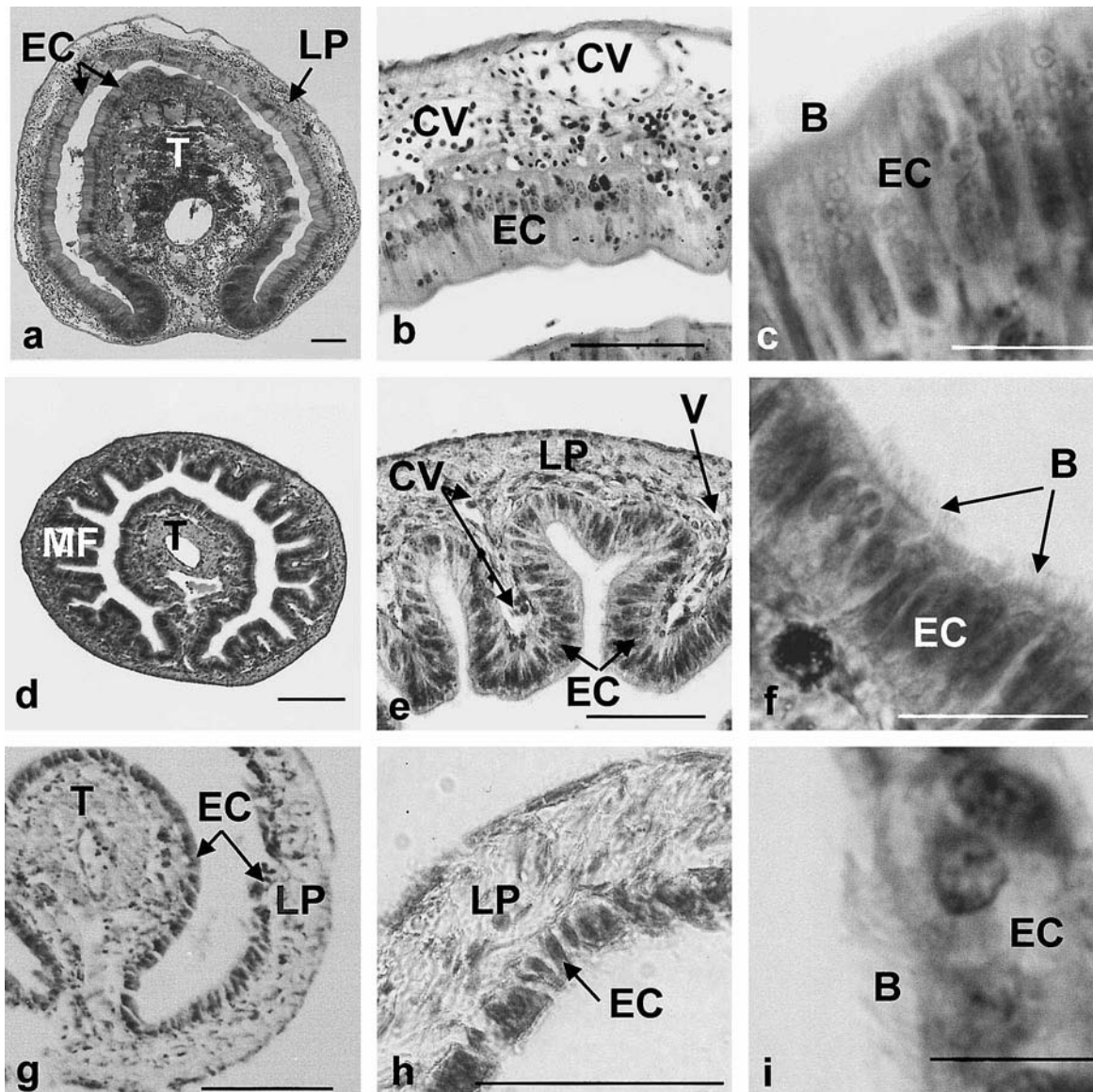
dered lamina propria mucosae (Figs. 2e–f, 3e).

In the maturation stage of *L. japonicum*, low in height mucosal folds and disorder both of mucosal epithelial cells arrangement and lamina propria mucosae were observed comparing with their counterparts in former stages (Fig. 1h–i). Mucosal folds were not obvious in the maturation stage of either of the nonparasitic-fluvial species, *L. kessleri* and the northern form of *L. reissneri* (Figs. 2g–i, 3f, g).

## DISCUSSION

In the present study, although each species showed a

similar intestinal structure at the larval stage, markedly different structures related to feeding / non-feeding were observed after the metamorphoses. Since brush borders comprising microvilli generally enhance the efficiency of the epithelium in its absorptive function, resulting from increasing the surface area of membrane (e.g. Bloom and Fawcett, 1968), their presence on epithelial cells infers an actively-absorptive condition in the latter. Accordingly, the presence of brush borders on the mucosal epithelial cells in the larval stages of all of the species examined in this study, as well as the metamorphosed and feeding adult stages of *L. japonicum*, should indicate active absorption by epithelial cells in these stages.

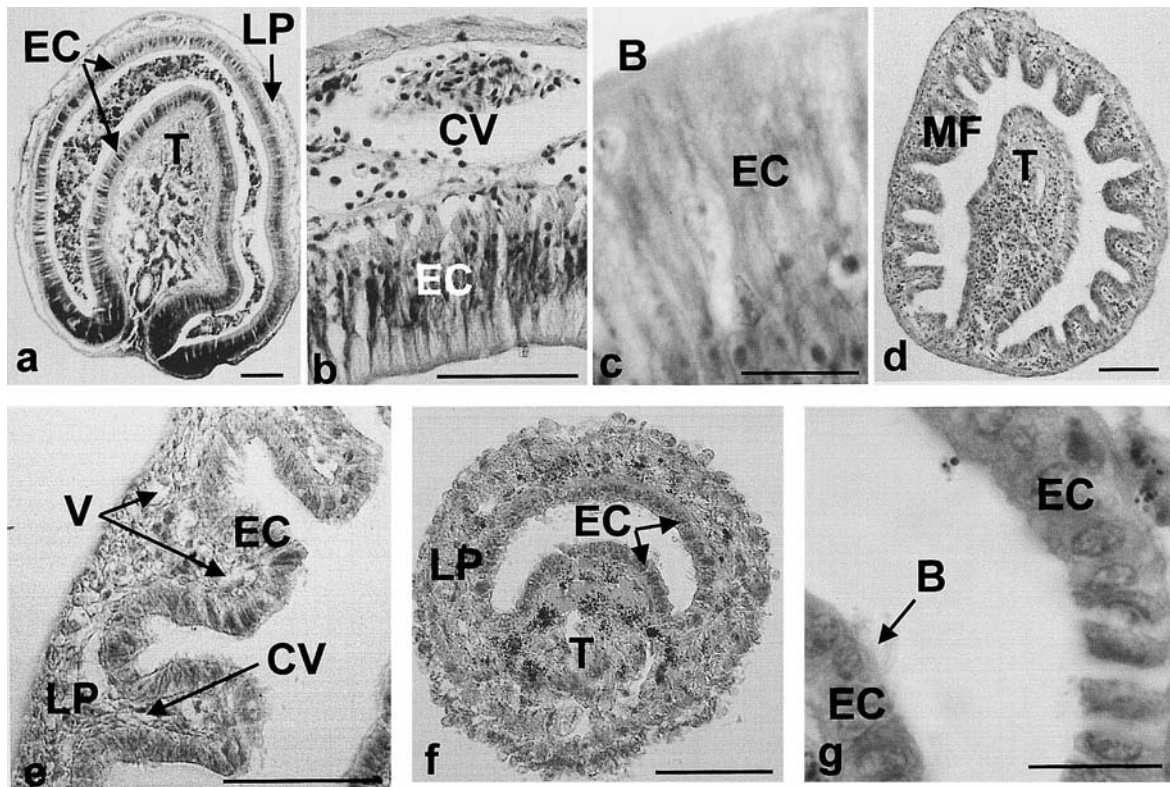


**Fig. 2.** Transverse sections of the posterior intestine of nonparasitic-fluvial species, *Lethenteron kessleri*. a; Larval stage. b; Enlargement of "a" showing mucosal epithelial cells and lamina propria mucosae. c; Enlargement of b showing mucosal epithelial cells. d; Metamorphosed stage. e; Enlargement of d showing mucosal fold. f; Enlargement of e showing mucosal epithelial cells. g; Maturation stage. h; Enlargement of g showing lamina propria mucosae. i; Enlargement of h showing mucosal epithelial cells. Scale bars = 50 µm, except c, f and i indicating 10 µm. Abbreviations as in Fig. 1.

Judging from the height, the three types, high, middle and low mucosal folds observed at metamorphosed and feeding adult stages in *L. japonicum* should correspond with primary, secondary and tertiary folds, respectively, proposed in developing order by Youson and Connelly (1978). Excluding typhlosole with high mucosal folds for the most part, *L. japonicum* in the present study revealed the regular pattern of arrangement in the three types of mucosal folds in opposition to some parasitic species previously reported, e.g. *Petromyzon marinus*, which have no definite pattern of arrangement (Youson and Connelly, 1978; Hilliard *et al.*, 1983), indicating the difference of developing patterns in mucosal folds among species. Mucosal folds appear to have resulted from an elevation of both the epithelium and the underlying connec-

tive tissue and muscle, which have commenced after metamorphosis stage 4 in parasitic *P. marinus* (Youson and Connelly, 1978). In the present study, low in height of mucosal folds observed at metamorphosed stage in the non-parasitic species were correspond in their proportion to those of tertiary folds at metamorphosed stages in parasitic *L. japonicum*, indicating the insufficient elevation caused by the later developmental onset or oppression of development in the former.

In both the metamorphosed and feeding adult stages of parasitic *L. japonicum*, the development of mucosal folds with apical pits over the entire inner wall of the intestine, including the typhlosole, should result in a very much greater absorptive surface area. Apical pits, which indicate the existence of



**Fig. 3.** Transverse sections of the posterior intestine of nonparasitic-fluvial species, the northern form of *Lethenteron reissneri*. a; Larval stage. b; Enlargement of "a" showing mucosal epithelial cells and lamina propria mucosae. c; Enlargement of b showing mucosal epithelial cells. d; Metamorphosed stage. e; Enlargement of d showing mucosal fold. f; Maturation stage. g; Enlargement of f showing mucosal epithelial cells. Scale bars = 50 µm, except c and g indicating 10 µm. Abbreviations as in Fig. 1.

secretory cells releasing the content of the granules, have been reported from only feeding stage of parasitic species, with an example of *P. marinus* (Youson, 1981). These characteristics indicate increase of nutrient absorption which enables more rapid growth during the parasitic period, as already reported for *P. marinus* (Youson and Connelly, 1978; Youson, 1981).

On the other hand, after the upstream migration stage of *L. japonicum* and metamorphosed stages of *L. kessleri* and the northern form of *L. reissneri*, brush borders were scarcely evident on the epithelial cells of the intestine, including the mucosal fold. Together with the reduced height of the latter and disordered lamina propria mucosae, such indicates that the cells had decreased or lost their absorptive capability (Battle and Hayashida, 1965; Youson, 1981). Vacuolations observed in upstream migration stage individuals of *L. japonicum*, as well as metamorphosed stage individuals of *L. kessleri* and the northern form of *L. reissneri*, suggested that the intestine had degenerated both functionally and structurally, according to the progress of maturation described in previous reports (Battle and Hayashida, 1965; Youson, 1981; Tsuneki and O uji, 1984).

In parasitic-anadromous *L. japonicum*, the development of extensive longitudinal mucosal folds with functional epithelial cells in the intestine at the metamorphosed stage may reflect an adaptive change for parasitic feeding, as well as

osmoregulation in a marine environment (Pickering and Morris, 1973; Youson and Connelly, 1978). On the contrary, nonparasitic-fluvial *L. kessleri* and the northern form of *L. reissneri* exhibited only shrunk mucosal folds appearing dysfunctional structures. Additionally, in a single lamprey population comprising of parasitic and nonparasitic forms of *Lampetra richardsoni*, the developmental mucosal folds had been observed at the former individuals on and after metamorphosed stage, while the latter individuals showed an atrophied intestine (Youson and Beamish, 1991). Owing to the absence of feeding just before metamorphosis in nonparasitic species (Hardisty and Potter, 1971a; Hardisty, 1986), the development of intestinal mucosal folds should be an unnecessary feature in metamorphosed nonparasitic lamprey species, representing that such may be rudiments of an ancestral parasitic species (Youson, 1981). Similar rudimentary development of intestinal mucosal folds for the nonparasitic lamprey species during the metamorphosis has been observed in the other generic (*Lampetra*) lamprey satellite species (Hilliard *et al.*, 1983).

Within the monophyletic group comprising *L. japonicum*, *L. kessleri* and the northern form of *L. reissneri*, the latter two species are paraphyletic, each being more closely related to *L. japonicum* with the different degree of genetic divergence than to the other nonparasitic species, based on allozyme data (Yamazaki and Goto, 1998). Accordingly, speciation of non-

parasitic *L. kessleri* and the northern form of *L. reissneri* may have occurred separately from ancestral stocks of parasitic *L. japonicum* during different geological periods. This supports a previously advocated direction of speciation in lamprey satellite species (Hubbs, 1925; Zanandrea, 1959; Hardisty and Potter, 1971b; Vladikov and Kott, 1979).

## ACKNOWLEDGMENTS

We thank Prof. emeritus F. Yamazaki and Dr. E. Yamaha, Hokkaido University, for their guidance in the course of this study and invaluable suggestions. We also thank Miss Y. Arakane, Chitose Salmon Museum, Miss H. Sugiyama, Nakagawa Aquatic Park and Mr. M. Kobayashi, Tokyo University of Agriculture, for providing samples. We are grateful to Dr. G. S. Hardy, Thames, New Zealand for his invaluable suggestions and great help with English. This study was supported by a grant from Nakayama Foundation for Human Science to Y. Yamazaki.

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(Received November 24, 2000 / Accepted July 19, 2001)



## Appendix

Developmental stages and sex, locality, number of samples examined, ranges of total length (sample number deposited) in three taxa of *Lethenteron*.

*L. japonicum*. Larval males, Uzura River, Hokkaido, 11, 78.2–181.7 mm; Larval females, Uzura River, Hokkaido, 15, 97.4–196.1 mm; Metamorphosed males, Uzura River, Hokkaido, 11, 164.1–194.5 mm; Metamorphosed females, Uzura River, Hokkaido, 11, 168.3–198.6 mm; Feeding adult males, Saru River, Hokkaido, 3, 215.2–271.4 mm (HUMZ 163369–163371); Feeding adult females, Saru River, Hokkaido, 1, 304.0 mm (HUMZ 163368), Chitose River, Hokkaido, 1, 169.8 mm; Upstream migration males, Ishikari River, Hokkaido, 5, 359.5–478.0 mm; Upstream migration females, Ishikari River, Hokkaido, 13, 335.0–478.0 mm; Mature males, Ohno River, Hokkaido, 2, 385.1, 399.2 mm; Mature females, Ishikari River, Hokkaido, 3, 387.9–435.5 mm, Ohno River, Hokkaido, 2, 352.5, 431.0 mm, Uzura River, Hokkaido, 1, 388.2 mm, Mogami River, Yamagata Pref., 3, 397.0–431.0 mm.

*L. kessleri*. Larval males, Maruman River, Hokkaido, 3, 123.3–131.0 mm; Larval females, Maruman River, Hokkaido, 14, 76.5–131.0 mm; Metamorphosed males, Sarakao-Makikin River, Hokkaido, 6, 128.1–163.8 mm; Metamorphosed females, Sarakao-Makikin River, Hokkaido, 12, 128.2–157.7 mm; Mature males, Abira River, Hokkaido, 3, 126.6–142.0 mm, Tarumae River, Hokkaido, 1, 140.8 mm; Mature females, Betsubetsu River, Hokkaido, 1, 147.0 mm, Nishikitappu River, Hokkaido, 1, 116.3 mm.

Northern form of *L. reissneri*. Larval males, Ushiwatari River, Yamagata Pref., 6, 110.4–136.5 mm; Larval females, Ushiwatari River, Yamagata Pref., 7, 101.2–137.2 mm; Metamorphosed males, Ushiwatari River, Yamagata Pref., 8, 102.5–134.2 mm; Metamorphosed females, Monbetsu River, Hokkaido, 3, 119.7–161.6 mm, Ushiwatari River, Yamagata Pref., 11, 123.0–154.0 mm; Mature males, Ushiwatari River, Yamagata Pref., 4, 111.0–129.2 mm; Mature females, Ushiwatari River, Yamagata Pref., 5, 99.3–127.8 mm.